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Seroprevalence of Parvovirus B19 NS1-Specific IgG in B19-Infected and Uninfected Individuals and in Infected Pregnant Women

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Parvovirus B19 is the causative agent of erythema infectiosum in children, but the virus is associated with an increasing range of different diseases. These include acute and chronic arthritis, hydrops fetalis in pregnant women, aplastic anemia, and thrombocytopenia. The host's immune response is directed against the viral structural proteins VP1 and VP2. This study investigated the presence of IgG against the viral nonstructural protein NS1 using Western blot. Serum panels from healthy individuals, B19-infected pregnant women, and various disease groups were tested. The disease groups included patients with symptoms that may be linked to parvovirus B19 infection. The results showed that IgG against the NS1 protein was present in 22% of healthy individuals with past B19 infection. In cases of persistent or prolonged B19 infections, the prevalence of NS1-specific antibodies was as high as 80%. It is concluded that NS1-specific IgG may be used as an indicator of chronic or more severe courses of parvovirus B19 infections. ***J. Med. Virol. 60:48–55, 2000.***

KEY WORDS: parvovirus B19; NS1-specific antibodies; antibody prevalence; hydrops fetalis

INTRODUCTION

Parvovirus B19 was discovered by chance in sera of healthy blood donors [Cossart et al., 1975]. Several years later, B19 infections were linked to erythema infectiosum (fifth disease), a common childhood exanthema [Anderson et al., 1983]. To date, B19 is the only member of the *parvoviridae* known to cause disease in humans. Infections occur in childhood and throughout adult life and about 70–80% of the population are seropositive by the age of 40 years [Cohen and Buckley, 1988]. Progress in the field of molecular biology led to the development of assays for the detection of viral nucleic acids in sera and biopsies, which allowed the association of B19 infections with more severe symptoms. Among these are hydrops fetalis [Anderson et al., 1988], acute and chronic arthritis [Reid et al., 1985; Naides et al., 1990; Biasi et al., 1996], acute and persistent aplastic anemia, thrombocytopenia, pancytopenia [Chorba et al., 1986; Young, 1988; Srivastava et al., 1990] and persistent infections in immunocompromised patients [Flunker et al., 1998] and also rarely in immunocompetent individuals [Cassinotti et al., 1993]. Recent case reports on B19-associated hepatitis [Hillingso et al., 1998; Pardi et al., 1998], myocarditis [Orth et al., 1997; Enders et al., 1998], and encephalitis [Umene and Nuone, 1995] have shown the increasing diversity of symptoms that can arise during B19 infections.

Because the symptoms of B19 infection are nonspecific and can therefore be confused with various other infectious and noninfectious agents, diagnosis relies on immunodiagnostic or DNA testing methods. Laboratory diagnosis is made routinely by enzyme-linked immunosorbent assay (ELISA), Western blot, or similar test formats using either or both of the structural proteins VP1 and VP2. In general, IgM antibodies against the structural proteins VP1 and VP2 are the first serological markers of an acute B19 infection. They may be detected 6–10 days after the first contact, whereas IgG antibodies can be detected about 12 days after infection. During the following weeks the concentration of IgM antibodies falls to an undetectable level, whereas IgG persists lifelong and may be used as a

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marker for past B19 infection. In addition to immunoglobulins against the capsid proteins VP1 and VP2, patients with persistent or chronic B19 infections may also develop immune reactions to the nonstructural protein NS1. NS1-specific IgG has been detected in sera from patients with chronic arthritis and other persistent B19 infections [von Poblotzki et al., 1995a, 1995b]. We tested a large panel of sera from patients with diverse symptoms associated with parvovirus B19 infections for the presence of NS1-specific antibodies. The seroprevalence of NS1-specific antibodies was investigated with respect to B19-associated symptoms.

METHODS

Serum Samples

A total of 250 serum samples from patients with different symptoms known to be associated with B19 infections were tested for the presence of NS1-specific antibodies. Samples were divided into groups, each group representing a distinct pattern of symptoms. All patients were grouped only once, even if symptoms allowed classification into more than one group. A total of 153 samples from healthy individuals were used as controls and were screened for the presence of B19-specific antibodies. Serum samples were generously provided by the Institute for Medical Microbiology, University of Regensburg, Germany; by the Swedish Institute for Infectious Disease Control, Stockholm, Sweden; by the Medizinisch-diagnostisches Gemeinschaftslabor, Stuttgart, Germany; by Dr. Louwen, Westfälische Wilhelms-Universität Münster, Münster, Germany; and by Prof. Bernhard Lang, Innere Medizin I, Universitätsklinik Regensburg, Germany.

Group 1: 153 healthy individuals with or without prior B19 infection. The healthly status of donors was verified by means of a 33-item questionaire developed by the clinical Department for Internal Medicine at the University of Regensburg.

Group 2: 43 acutely B19-infected, immunocompetent individuals, either with asymptomatic B19 infections or suffering from erythema infectiosum or flu-like symptoms. Acute infections were diagnosed with respect to positive IgM values.

Group 3: 39 pregnant women with B19 infections who were diagnosed following contact with infected persons or following the occurrence of typical B19-associated symptoms in the mother or fetus.

Group 4: 22 patients suffering from hemapoietic disorders such anemia, aplastic crisis, thrombocytopenia, and pancytopenia. Seven of these patients presented with liver dysfunction in combination with severe aplastic anemia.

Group 5: 22 patients with diverse joint symptoms such as acute or chronic arthritis, arthralgias, polyarthritis, or synovitis of unknown origin.

Group 6: 66 patients with rheumatoid arthritis (RA) or juvenile RA. RA was classified in accordance with the criteria of the American Society of Rheumatology (http://www. rheumatology.org/classifi/classifi.html).

Group 7: 42 patients with systemic lupus erythematosus (SLE) characterized by the presence of antinuclear antibodies (ANA).

Group 8: 11 B19-infected patients with immunosuppression due to non-Hodgkin lymphoma, acute myeloid leukemia (AML), or acute lymphoid leukemia (ALL). B19 infections were verified by positive IgG and IgM values and by the detection of B19 genomes in the sera using polymerase chain reaction (PCR).

Group 9: 5 chronically B19-infected, immunocompetent patients, with diverse symptoms such as long-lasting joint symptoms, hemapoietic disorders, or recurrent exanthema. The persistence of infection was documented by the presence of B19 genomes in sera or bone marrow samples taken over a period of at least 6 months.

Detection of B19-Specific Antibodies and DNA

IgM and IgG against VP1, VP2, and NS1 proteins of parvovirus B19 were detected in sera by Western blot assays according to the manufacturer's instructions (Recomblot, Mikrogen GmbH; Munich, Germany). The test system is based on viral proteins expressed in *Escherichia coli*. The purified protein preparations have been shown to react specifically and do not exhibit cross-reactivities with bacterial proteins. Additionally, the B19 IgM and IgG status of these sera were confirmed using a VP2-based ELISA (Biotrin, Dublin, Ireland).

Detection of B19 genomes was made using nested-PCR amplification of the region from nt 2956 to 3448 of the B19 genome (nucleotide positions refer to the isolate pYT103 [Shade et al., 1986]). PCR was carried out as described previously [Hemauer et al., 1996].

RESULTS

Prevalence of NS1-Specific IgG in Healthy Individuals With Past B19 Infection

To examine whether NS1-specific antibodies may also be used as a marker for other courses of B19 infection, 250 sera were tested from patients with various symptoms for the presence of NS1-specific IgG. These patients displayed symptoms known to be associated frequently or rarely with B19 infections. The patients were divided into nine groups with respect to symptoms to analyze the prevalence of NS1-specific antibodies in relation to the various B19-associated diseases.

Sera derived from 153 healthy individuals (age range 18–75 years) with or without past B19 infection were also tested. All the sera were selected from persons who answered a 33-item questionnaire confirming their healthy status. None exhibited or could remember having had B19-associated symptoms. The results of the seroprevalence for NS1-specific IgG in healthy individuals are shown in Table I. All the sera were IgM negative by ELISA. When tested for IgG against the viral capsid proteins by ELISA and Western blot, 80% (123 sera) were positive. Of these, 27 (22%) were also positive for NS1-specific IgG by Western blots (see Table I). None of the sera in this group were found to

TABLE I. Seroprevalence of Parvovirus B19 IgG in Healthy, Pregnant and Disease Groups

Manifestation	Number of patients	Number (percentage) of patients with IgG against VP1/VP2	Number (percentage) of patients with IgG against NS1
Group 1: Healthy population	153	123 (80%)	27 (22%)
Group 2: Acute infection in immunocompetent (asymptomatic, erythema infectiosum, flu-like symptoms)	43	43 (100%)	5 (11%)
Group 3: Pregnant women with contact with B19-infected persons or displaying B19 associated symptoms (exanthema, flu-like, hydrops fetalis)	40	39 (97%)	24 (61%)
Group 4: Hematopoeitic disorders (anemia, aplastic crisis, thrombocytopenia, pancytopenia)	22	21 (95%)	10 (47%)
Group 5: Joint symptoms (acute and chronic arthritis, arthralgias, polyarthritis, synovitis)	22	21 (95%)	10 (47%)
Group 6: Rheumatoid arthritis, juvenile rheumatoid arthritis	66	46 (70%)	13 (28%)
Group 7: Systemic lupus erythematosus	42	34 (81%)	13 (38%)
Group 8: Chronic infection in immunocompromised with non-Hodgkin lymphoma, AML, or ALL	11	11 (100%)	6 (54%)
Group 9: Chronic infection in immunocompetent (at least 6 months PCR positive)	5	5 (100%)	4 (80%)

AML, acute myeloid leukemia; ALL, acute lymphoid leukemia; PCR, polymerase chain reaction.
Antibody reactivity was tested against viral structural (VP1/VP2) and nonstructural (NS1) proteins.

contain B19 DNA when tested by nested PCR. Of the 153 individuals, 30 were negative for VP1/VP2-specific IgG and correspondingly none of them had IgG or IgM against the NS1 protein.

Prevalence of NS1-Specific IgG in Patients With Symptoms Associated With B19 Infection

Sera derived from 43 patients infected acutely with parvovirus B19 (group 2) were tested for the presence of NS1-specific IgG and IgM. All sera displayed IgM antibodies against the structural proteins VP1 and VP2 when analyzed by Western blots and VP2-based ELISA verifying the acute nature of the infection; in addition, detectable amounts of VP1/VP2-specific IgG were demonstrated in all sera. This finding probably indicates that the patients had been infected at least 12 days before the serum samples were obtained. The infections proceeded either as asymptomatic or with the classical B19-associated manifestations such as erythema infectiosum or flu-like illness. None of the sera were positive for NS1-specific IgM, five of these patients (11%) showed NS1-specific IgG. A correlation of the NS1-specific IgG with either asymptomatic infections or with erythema infectiosum was not observed. The prevalence of NS1-specific IgG in acute infection was only half of that found in healthy persons (group 1, see Table I).

Parvovirus B19 is known to cause severe complications during pregnancy due to intrauterine infection of the fetus, particularly during the second and third trimester of pregnancy [de Krijger et al., 1998; Essary et al., 1998]. Fetal infection may be followed by hydropic changes due to the destruction of erythroid precursor cells in the fetal liver. We tested the sera of 40 pregnant women (group 3) in whom diagnosis was made following contact with infected persons or who presented with typical symptoms associated with B19 infections (exanthema, fever) or with sonographic abnormalities of the fetus. In addition, other viral infections such as cytomegalovirus (CMV), varicella zoster virus, and rubella, which are known to cause hydropic changes, had been excluded by routine diagnosis except for one woman who was acutely infected with CMV and negative for parvovirus B19. VP1/VP2-specific IgG was detected in 39 sera (97%), 33 of the patients were positive for VP1/VP2-specific IgM and 24 of the pregnant women displayed IgG against the NS1 protein (61%; Table I). NS1-specific IgM could not be detected in any of the sera. In 23 women, hydropic changes of the fetus were observed, e.g., hydrops fetalis, fetal ascites, pericardial effusion, or fetal encephalitis. All women with sonographic abnormalities had serological markers for an acute B19 infection, as either IgM or PCR or both were positive. NS1-specific IgG could be detected in 18 of the 23 women with B19-associated hydropic changes in the fetus (78%). In 16 uncomplicated pregnancies the prevalence was significantly lower (37%), with only 6 of these women displaying IgG against NS1 (Table II).

Infections with parvovirus B19 are frequently accompanied by hemapoietic disorders such as anemia or aplastic anemia due to the destruction of erythroid precursors in the bone marrow [Kurtzman et al., 1987]. Severe thrombocytopenia and pancytopenia have also been reported to be caused by B19 infection [Young, 1988; Brown and Young, 1995]. We therefore included a panel of 22 sera from patients with anemia, thrombocytopenia, or pancytopenia in this study (group 4). Seven of these patients developed anemia-associated liver dysfunctions due to the severe aplastic crisis [Yoto et al., 1994; Langnas et al., 1995]. The IgG results are shown in Table I. Of 21 patients (95%) who were found to be positive for VP1/VP2-specific IgG, 10 (47%) had an IgG response to NS1 protein. Five of the VP1/VP2-

TABLE II. Seroprevalence of Parvovirus B19 IgG and IgM to Structural (VP1/VP2) and Nonstructural (NS1) Proteins in Pregnant Women Infected With Parvovirus B19

Manifestation	Number of cases	Number (percentage) of women with IgM against VP1/VP2	Number (percentage) of women with IgG against VP1/VP2	Number (percentage) of women with IgG against NS1
Pregnant women	39	33 (85%)	39 (100%)	24 (61%)
Without complications during pregnancy	16	14 (88%)	16 (100%)	6 (37%)
With hydropic changes of the fetus	23	21 (91%)	23 (100%)	18 (78%)

and NS1-positive sera had detectable IgM titers against the capsid proteins, and viral DNA was detected using PCR, indicating a direct correlation between the symptoms and an acute B19 infection. None of the sera were positive for NS1-specific IgM.

The involvement of joint symptoms has been reported to be associated frequently with B19 infections especially in adults, whereas exanthema is the most frequent manifestation in children [Naides et al., 1990; Biasi et al., 1996; Phillips, 1997]. Of 22 patients with diverse joint symptoms of unknown origin such as acute or chronic arthritis, arthralgia, polyarthritis, or synovitis (group 5), 21 (95%) displayed VP1/VP2-specific IgG and 10 of these (47%) had IgG against the NS1 protein. The sera from patients with acute arthritis and synovitis were positive for VP1/VP2-specific IgM. Again all of the sera were negative for NS1-specific IgM. The results for group 6 are shown in Table I. These sera were derived from a total of 66 patients with RA or juvenile RA, a manifestation that has also been linked to B19 infection [Mimori et al., 1994; Takahashi et al., 1998]. In contrast to those in group 5, who displayed diverse joint symptoms of unknown origin, all patients fulfilled the criteria of the American Society of Rheumatology for the classification of RA, i.e., they were rheumatoid factor positive, indicating an autoimmune reaction against gamma globulin. VP1/VP2-specific IgG was detected in 46 (70%) of these 66 RA patients and 13 showed an IgG response against the NS1 protein (28%; Table I). These samples were negative for both NS1- and VP1/VP2-specific IgM and B19 DNA. The percentage of NS1-positive sera in patients with RA is only slightly higher than that of healthy individuals with past B19 infection (22%) and clearly lower than that found in patients with acute joint inflammation (47%, group 5).

Parvovirus B19 has also been linked occasionally to SLE [Fawaz-Estrup, 1996; Nigro et al., 1997]. We included 42 sera derived from SLE patients in this study. The sera of 34 patients (81%) contained VP1/VP2-specific IgG and were free of IgM against the capsid proteins, indicating the status of past B19 infection. NS1-specific IgG was detectable in 13 of the seropositive patients (Table I). This finding reflects a slightly elevated prevalence of 38% for NS1-specific immune reaction when compared with the group of healthy individuals (22%). When the sera were examined by PCR, only two had detectable B19 DNA. In one of these patients, SLE symptoms were enhanced by the B19 infection. This 26-year-old female patient, who had documented SLE for 3 years, was seronegative for parvovirus B19 and became infected after her daughter developed erythema infectiosum. Serological diagnosis showed VP1/VP2-specific IgM and a strong positive PCR signal followed by the development of IgG antibodies. Viral DNA was detectable in the sera for 6 weeks after the onset of symptoms. NS1-specific IgG were first detected about 3 weeks after the appearance of VP1/VP2-specific IgG. In this case parvovirus B19 was not the causative agent for SLE, but probably a trigger for the clinical manifestation.

In chemotherapeutically immunosuppressed tumor patients, persistent B19 infection may become established following acute infection [Flunker et al., 1998]. We tested the sera from 11 patients with non-Hodgkin lymphoma, AML, or ALL (group 8). All sera were VP1/VP2-IgG- and IgM-positive (100%). B19 DNA was detected by PCR in sera from 10 of the patients (91%). Because viral DNA was detected in consecutive sera sampled over a period of several months, this was a clear indication that persistent B19 infections had been established in these immunosuppressed tumor patients. In 6 patients (54%), the presence of NS1-specific antibodies was demonstrated (see Table I); 5 of these were PCR positive.

Persistent B19 infections may also occur rarely in immunocompetent individuals [Cassinotti et al., 1993]. In cases in which B19 DNA could be shown to persist either in sera or bone marrow for at least 6 months, these immunocompetent individuals were classified as chronically infected with parvovirus B19 (group 9). The symptoms associated with these persistent courses of B19 infection varied from long-lasting joint symptoms to weak hemapoietic disorders such as anemia and thrombocytopenia or recurrent exanthema. Only 5 immunocompetent patients displayed the criteria of persistent B19 infection. The results for group 9 are shown in Table I. All of them (100%) had IgG antibodies against B19-capsid proteins and 4 (80%) also showed NS1-specific IgG, indicating a close correlation of NS1-specific immune reaction with chronic forms of B19 infection due to the strongly elevated prevalence as compared with healthy individuals (22%).

Time Course of the Appearance of NS1-Specific Antibodies

In cases in which consecutive serum samples taken from one individual were available, NS1-specific IgG occurred later than IgG for VP1 and VP2. Table III shows four examples of antibody development in B19-infected patients with different symptoms. All patients were positive for B19 DNA over weeks and months af-

TABLE III. Occurrence of Antibodies to Parvovirus B19 Structural (VP1/VP2) and Nonstructural (NS1) Proteins From Four Patients

Patient	Symptoms	Serum donation date	VP2-specific IgM	VP1/VP2-specific IgG	NS1-specific IgG	PCR
I	Aplastic crisis associated with liver dysfunction	January 25	–	–	–	+
		February 28	+	+	–	+
		March 3	+	+	+	+
II	Immunosuppressed Acute lymphoblastic leukemia	April 9	–	–	–	–
		June 6	+	+	–	+
		July 5	–	+	(+)	+
		July 26	–	+	+	+
III	Aplastic anemia	January 11	+	+	–	+
		January 25	+	+	–	+
		February 2	+	+	(+)	+
		March 3	+	+	+	+
		April 24	+	+	+	+
		October 7	–	+	+	+
		December 13	–	+	+	+
IV	Pregnant women with hydrops fetalis	April 24	+	–	–	+
		May 8	+	+	–	+
		May 13	+	+	(+)	+
		July 10	–	+	+	–

Sera were donated on different dates and were also tested for B19 DNA by polymerase chain reaction (PCR).

ter infection as shown by PCR. Cases I and III are immunocompetent subjects with no known underlying disease. Case II is an individual immunosuppressed due to chemotherapy for ALL and case IV is a B19-infected women with hydropic complications during pregnancy. In all cases for which consecutive sera were available, NS1-specific IgG became detectable 4–6 weeks after the first contact with the virus and can therefore be detected about 2 weeks after the onset of IgG against the capsid proteins. We were not able to demonstrate IgM against the nonstructural protein and NS1-specific IgG was detectable only in combination with IgG against the structural proteins VP1 and VP2. This clear connection to B19 infection demonstrates that the detection of NS1 antibodies is a specific reaction (Table III).

DISCUSSION

Parvovirus B19 may still be considered to belong to the group of emerging viruses. During the past few years it has been shown that parvovirus B19 infections may be associated with an increasingly varied panel of rare, rather untypical symptoms in addition to the more commonly observed manifestations such as erythema infectiosum, hydrops fetalis, and aplastic anemia. Due to this diversity of symptoms related to infections with parvovirus B19, a reliable differential diagnosis has become increasingly important. Earlier reports had shown that the presence of antibodies against the nonstructural protein NS1 of parvovirus B19 could be linked to chronic or persistent forms of infection [von Poblotzki et al., 1995a, 1995b]. To examine whether NS1-specific antibodies may also be used as a marker for other courses of B19 infection, 250 sera were tested from patients with various symptoms for the presence of NS1-specific IgG. These patients displayed symptoms that are known to be associated frequently or rarely with B19 infections. With respect to the symptoms the patients were placed into nine groups to analyze the prevalence of NS1-specific antibodies in relation to the various B19-associated diseases.

Before attempting to demonstrate an association between the NS1-specific immune reaction and particular B19-correlated symptoms in pregnancy and various disease states, a panel of sera derived from healthy individuals was tested for the presence of NS1-specific IgG. The IgG seroprevalence for NS1 in the healthy population was 22%, which is in accordance with recent data obtained by studying healthy blood donors using Western blots [Searle et al., 1998; Venturoli et al., 1998]. Von Poblotzki and et al. [1995a, 1995b] found the seroprevalence in the general population to be lower. This finding may have been due to the larger serum panels tested in the more recent studies and to the use of Western blots instead of a less sensitive ELISA. In addition it should be considered that the healthy individuals tested in the current study were tested retrospectively and therefore the values can reflect only the status of past B19 infection and not the course and the symptoms the patients actually had. Most of the healthy individuals were not able to recall the event of the previous B19 infection or any of the manifestations of the disease. In comparison, the seroprevalence of NS1-specific IgG was found to be elevated in the groups with prolonged or persistent parvovirus B19 infections. These patients presented with arthritis and various joint symptoms, or hematopoietic disorders such as anemia with or without liver failure, thrombocytopenia, or pancytopenia. In all cases the symptoms could be correlated with a previous B19 infection.

Prolonged viremia in infected individuals may lead to the infection of cells other than the usual erythroid

precursors. Such cells are unable to support a productive infection cycle and therefore do not allow the production of viral particles. Gene expression in nonpermissive cells is shifted toward the preferential transcription of the NS1 gene without production of the capsid proteins VP1 and VP2 [Liu et al., 1992; Pallier et al., 1997]. The NS1 protein has been shown to be cytotoxic [Ozawa et al., 1988] and is able to the stimulate apoptotic processes [Moffatt et al., 1998], resulting in cell lysis and the release of NS1 protein, a process that may render this nonstructural viral component accessible to the immune response of the host. The infection of thrombocytes, reticulocytes, neutrophils, and other white blood cells is associated with symptoms such as thrombocytopenia, neutropenia, or pancytopenia due to the continuous destruction of these cells. That prolonged viral persistence in the individual may be a precondition for the formation of NS1-specific antibodies as supported in this study by the fact that there is reduced incidence of IgG in acutely infected persons with erythema infectiosum compared with the healthy population. In patients with acute B19 infection, the seroprevalence of NS1-specific immune reactions (11%) is only half that of the group of healthy individuals (22%) and exceptionally lower than that observed in patients with persistent B19 infections (Table I). This finding may be explained by the fact that the virus is not present long enough to stimulate the production of NS1-specific antibodies. When testing consecutive sera derived from patients with chronic B19 infections it was shown that NS1-specific IgG occur several weeks after IgG produced against the viral capsid proteins (Table III). This finding supports the hypothesis that viral persistence or at least inefficient virus elimination has to be established after acute infection before NS1 proteins are synthesized in elevated concentration and become accessible to the host's immune system.

NS1-specific immune reactions were observed in 61% of the pregnant women infected with parvovirus B19. This observation may be due to a low level immunosuppression generally found during pregnancy, which may lead consequently to ineffective virus elimination. With respect to the cases that simultaneously displayed hydropic manifestations in the fetus, NS1-specific IgG was detectable in 78% of the women's sera (Table II). This may also be used as an indication that prolonged B19 persistence during pregnancy may enhance the risk of fetal infection and disease. Because fetal complications occur 2–4 weeks after maternal infection and antibodies against the NS1 protein first become detectable around 4–6 weeks postinfection, we cannot exclude the possibility that this may contribute to the higher frequency of NS1 IgG detected in sera from women with fetal complications. This hypothesis is supported by Searle et al. [1998], who did not find significant differences in the prevalence of NS1 IgG in pregnant women with and without fetal complications, but also detected a higher prevalence of NS1-specific antibodies in B19 infections during pregnancy. Further investigations with a larger number of patients will help to further elucidate this point.

Virus infections are associated commonly with anemia, but also with thrombocytopenia, neutropenia, and pancytopenia. NS1-specific antibodies were found in 47% of patients with hematological disorders, which is about double that observed in healthy controls (see Table I). In addition to VP1/VP2- and NS1-specific IgG, five of the patients displayed IgM against the viral capsid proteins with simultaneous presence of B19 DNA. It may be concluded that these patients had been infected recently with parvovirus B19 and that the virus had not been eliminated from the organism although elevated IgG levels were found. As thrombocytes, reticulocytes, and neutrophils do not represent target cells for productive B19 infection, it can also be concluded that in these cases nonpermissive cells had been infected due to prolonged viral presence in the host followed by the synthesis of NS1 protein.

Of the patients with joint symptoms of unknown origin, 47% showed an antibody reaction against the NS1 protein, which is a significantly higher percentage than reactions observed in healthy individuals or with erythema infectiosum (see Table I). Because not all of the patients showed IgM or viral DNA in their sera, the symptoms can be mediated either immunologically or through direct cytotoxic viral action in cases in which the virus is still present. B19 infections have been linked to a variety of autoimmune reactions [Soloninka et al., 1989; Vigeant et al., 1994; Kerr and Boyd, 1996]. The joint symptoms may be caused by immune complexes between antibodies and virus proteins or particles present in the synovial fluid. These complexes may evoke autoimmune reactions via complement activation or cytokine secretion by immunologically active cells. Furthermore, it cannot be excluded that due to genomic variation B19 mutants exist that display epitopes mimicking autoantigens and therefore induce an autoimmunological process [Lunardi et al., 1998]. Alternatively, the joint destruction may also be caused directly by the B19 infection. Recent data indicate that parvovirus B19 may persist in synovial membranes of persons with arthritic symptoms [Cassinotti et al., 1998; Stahl et al., 1998]. Therefore, it seems reasonable that low levels of virus replication in this nonpermissive tissue associated with the production of NS1 proteins may contribute to an continuous cell destruction followed by inflammatory reactions.

In contrast, the prevalence of NS1-specific antibodies in RA patients is only slightly elevated (28%) when compared with healthy controls (22%) (see Table I). In addition, these patients did not display any serological markers indicating an acute B19 infection, because neither VP1/VP2-specific IgM nor viral DNA could be detected. This result confirms recent findings that RA is not associated with acute or persistent parvovirus B19 infections [Kerr et al., 1996], although there are reports of individual cases in which an association between RA and B19 infection has been described [Mimori et al., 1994; Takahashi et al., 1998]. Because

symptoms similar or identical to rheumatic manifestations are common side effects of B19 infections, RA-enhancing mechanisms cannot be excluded. Similar mechanisms have been proposed for the development of SLE. Data describing an association of B19 infection and SLE have been published [Fawaz-Estrup, 1996; Nigro et al., 1997] along with data that discount it [Nesher et al., 1995]. When testing sera derived from SLE patients relative to the control groups, we detected a slightly increased prevalence of 38% of NS1-specific IgG (see Table I). In one case of SLE, however, the symptoms were enhanced when the patient was infected with parvovirus B19 [Hemauer et al., 1999]. Viral DNA could be detected in the consecutive sera of this patient over 6 weeks, reflecting incomplete virus elimination, and IgM remained positive over the complete follow up time (10 months), indicating an ineffective Ig-class switch.

The highest incidence of NS1-IgG-positive individuals was found in the persistent B19 infections in immunosuppressed (54%) and immunocompetent (80%) individuals (Table I). In all cases the presence of the virus over several months was confirmed using PCR either in serum or bone marrow samples, indicating the clear correlation between the prolonged persistence of parvovirus B19 and the formation of NS1-specific antibodies. These patients show a variety of B19-correlated symptoms such as chronic arthritis, chronic or recurring arthralgias, recurring exanthema, chronic anemia, thrombocytopenia, or pancytopenia. Furthermore, we observed a strongly elevated prevalence of NS1-specific IgG in pregnant women with complications during pregnancy (see Table II).

In conclusion, elevated levels of NS1-specific antibodies were found in patients with distinct B19-correlated manifestations. This phenomenon was particularly pronounced in persistent courses of B19 infection, independent of the host's immune status, in patients with joint symptoms or hemapoietic disorders (see Table I), and in pregnant women with B19-associated manifestations of the fetus (see Table II). Although there is no correlation between the appearance of NS1-specific antibodies and particular clinical manifestations, NS1-specific immune reactions may be used as a serological marker for persistent B19 infections. Because the number of patients tested in some groups was relatively small due to the rarity of the manifestation, the data may not be significant in some cases.

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